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Ocean warming and acidification affect the nutritional quality of the commercially-harvested turbinid snail *Turbo militaris*

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ABSTRACT

Rising levels of atmospheric carbon dioxide are driving ocean warming and acidification. This could cause stress resulting in decreases in nutritional quality of marine species for human consumption, if environmental changes go beyond the optimal range for harvested species. To evaluate this, we used ambient and near-future elevated temperatures and pCO_2 to assess impacts on the proximate nutritional composition (moisture, ash, protein, and lipids), fatty acids and trace elements of the foot tissue of *Turbo militaris*, a commercially harvested marine snail from south-eastern Australia. In a fully orthogonal design, the snails were exposed to ambient seawater conditions (22 ± 0.2 °C, pH 8.13 ± 0.01–450 µatm pCO_2), ocean warming (25 ± 0.05 °C), pCO_2 ocean acidification (pH 7.85 ± 0.02, ~880 µatm pCO_2) or a combination of both in controlled flow-through seawater mesocosms for 38 days. Moisture, ash, protein and total lipid content of the foot tissue in the turban snails was unaffected by ocean warming or acidification. However, ocean warming caused a reduction in healthful polyunsaturated fatty acids (PUFA) relative to saturated fatty acids (SFA). Under future warming and acidification conditions, there was a significant 3–5% decrease in n–3 fatty acids, which contributed to a decrease in the n–3/n–6 fatty acid ratio. The decrease in n–3 PUFAs, particularly Eicopentanoic acid (EPA), is a major negative outcome from ocean warming, because higher n–3/n–6 ratios in seafood are desirable for human health. Furthermore, ocean warming was found to increase levels of zinc in the tissues. Calcium, iron, macroelements, microelements and the composition of toxic elements did not appear to be affected by ocean climate change. Overall, the major impact from ocean climate change on seafood quality is likely to be a decrease in healthy polyunsaturated fatty acids at higher temperatures.

1. Introduction

Coastal marine ecosystems provide numerous ecological and socio-economic services to humans (Harley et al., 2006), including food and export income for local communities from harvesting wild populations of fish and invertebrates (FAO, 2012). The value of these resource are currently under threat from a variety of human-induced stressors (e.g. over exploitation, pollution, habitat degradation and invasive species), including ocean warming and acidification (Harley et al., 2006; Hoegh-Guldberg and Bruno, 2010; IPCC, 2014; Raupach and Fraser, 2011). As reported by the IPCC (2014), the average global sea surface temperature (SST) has already increased by 0.65–1.06 °C, over the period 1880 to 2012. Future SSTs are predicted to increase up to 4 °C by 2100 (IPCC, 2014). Furthermore, average seawater pH is predicted to decrease by a further 0.3–0.5 units, as the partial pressure of CO_2 reaches 800 ppm (Harvey et al., 2013; IPCC, 2007). These rapid ongoing changes in oceanic conditions are anticipated to cause stress to marine ecosystems, by impacting the physiology, reproductive success and survivorship of species, resulting in changes to species distribution and community structure (Helmuth et al., 2005; Pörtner and Knust, 2007; Pörtner, 2008; Przeslawski et al., 2008; Kroeker et al., 2013). The effects of climate change stressors on population ecology and species physiology has potential implications for both the quantity and quality of future seafood harvests.

Marine molluscs are a major component of world's fisheries (Leiva and Castilla, 2002). In 2013, the commercial harvest of marine molluscs was at least 9.8 million tonnes (FAO, 2015). Molluscs are often considered a delicacy and are known to be a nutritious source of protein, high-quality polyunsaturated fatty acids and essential minerals (Ab Lah et al., 2017a; Srilatha et al., 2013). Turban snails have been described

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as being among the best gastropods for human consumption in Australia (Yearsley et al., 1999) and are highly valued as food throughout Asia (Chen et al., 2004; Mason et al., 2014). Biochemical studies have confirmed that Turbinidae are a healthful food source, with comparable protein and fatty acid composition to other commercial species such as abalone (Mclachlan and Lombard, 1980; Freiji and Awadh, 2010; Mason et al., 2014; Ab Lah et al., 2017a). Currently the Turbinidae are only about 2% of the world gastropod catch, with a commercial harvest of around 8500 tonnes in 2013 (FAO, 2015). However, the demand for global fisheries product is increasing every year as the human population grows (Diana, 2009; Merino et al., 2012), leading to exploitation of new stocks (Dey, 2015). Relatively slow growing, large, gastropods that are not currently produced by aquaculture, such as the Turbinidae, are at risk from the combined pressures of over-exploitation and ocean climate change.

Ocean warming and acidification, might have negative impacts on the physiology of marine molluscs, which in turn could affect the biochemical properties and potential health benefits associated with the consumption of molluscs (Anacleto et al., 2014; Valles-Regino et al., 2015; Tate et al., 2017). Temperature has been identified as a driver of seasonal changes in the biochemical composition of the turbinid *Turbo brunneus* (Ramesh and Ravichandran, 2008) and other molluscs such as *Haliotis diversicolor* (Chiou et al., 2001), hybrid Tiger abalone (*H. laevigata* x *H. rubra*) (Mateos et al., 2010), *Pecten maximus* (Pazos et al., 1997), *Magallana gigas* (Dridi et al., 2007; De la Parra et al., 2005), *Crassostrea tulipa* (Yankson et al., 1991–1996) and *Pinctada radiata* (Gokoglu et al., 2006). These studies cannot separate the effects of temperature from other seasonal variables, such as food availability, however, in a manipulative experiment investigating the effect of elevated temperature on the biochemical composition of the clams *Ruditapes decussatus* and *R. philippinarum*, Anacleto et al. (2014) found that elevated temperature significantly decreased the relative proportion of some fatty acids. Elevated water temperature has also been shown to increase the total lipids and saturated fatty acids, but decrease protein, carbohydrates and unsaturated fatty acid contents in the filter-feeding Pacific oyster, *M. gigas* (Flores-Vergara et al., 2004). A manipulative ocean climate change study on the carnivorous marine gastropod *Dicathais orbita*, found that total protein in the flesh decreased by as much as 45% after 35 days of exposure to the combined effects of elevated temperature and pCO_2 acidification (Tate et al., 2017). Furthermore, there was a decrease in total lipids and a significant reduction in the relative proportion of n–3 and n–6 PUFAs in the foot tissue of these predatory whelks under future ocean conditions (Valles-Regino et al., 2015).

Overall there have been only a few studies investigating the impacts of climate change on seafood quality and no manipulative experiments have investigated the interaction of ocean warming and acidification on nutritional quality in herbivorous marine gastropods. This study therefore aimed to assess the effects of current conditions and ocean warming and acidification on the biochemical composition, fatty acid profiles and trace elements of edible tissue from *Turbo militaris*. *T. militaris* is the largest Australian turbinid and inhabits the shallow subtidal and low intertidal areas of rocky reefs, feeding on macroalgae (Smoothey, 2013). They occur from south-eastern Queensland to southern New South Wales, Australia (Beechey, 2004) in part of the coast identified as an 'ocean climate change hotspot', experiencing faster-than-average ocean warming (Hobday and Pecl, 2013). Although there is only a small scale commercial turban snail fishery in New South Wales (< 10 tons per annum, Rowling et al., 2010), *T. militaris* is subject to substantial recreational harvest (Cooling and Smith, 2015). The outcomes of this study will improve our understanding of the implications of ocean climate change for seafood quality in a commercial and recreationally valued gastropod.

2. Materials and methods

2.1. Study site and experimental design

A 38 day experiment was undertaken in 12 outdoor mesocosms (1100 L, 135 cm diameter x 90 cm high) at the National Marine Science Centre (NMSC), Coffs Harbour, Australia (30° 16′3.70″S, 153° 8′15.31″E). A total of 144 individuals of *T. militaris* were collected from rock platforms around Coffs Harbour and 12 snails were randomly added to each mesocosm. The experiment involved four treatments arranged in an orthogonal design with current (22 °C ± 0.20) and future (25 °C ± 0.045) ocean temperatures and current (393 ± 12 ppm; pH = 8.13 ± 0.01) and future (830 ± 48 ppm; pH = 7.85 ± 0.02) ocean pCO_2 (n = 3 mesocosms per treatment). Current seawater temperature and pCO_2 were based on SST averages for the Coffs Harbour coast between September to November 2014 (Tate et al., 2017). Future ocean conditions were based on predictions for 2100 using the IPCC model RCP 8.5 (IPCC, 2013).

Seawater was pumped in from the adjacent open ocean, passed through a 50 μm filter and supplied to each mesocosm at 3 L/min. The temperature of seawater in the mesocosms was maintained by heater/chiller units (Aquahort Ltd., Auckland, New Zealand). Future pCO_2 treatments were generated by bubbling CO_2-enriched air into each mesocosm via a gas mixer (PEGAS 4000 MF, Columbus Instruments, CO, USA), while the current pCO_2 treatments were bubbled with ambient air. Water temperature and pH were measured daily in each mesocosm using a Hach HQ40d multi probe. The pH probe was calibrated daily using NIST buffers with readings subsequently converted to total scale (pHT) following methods outlined in SOP6a (Dickson et al., 2007). Total alkalinity (AT) for the system was measured on 25 days during the study using Hg fixed and filtered (40 μm) water samples and a potentiometric titration (888 Titrando, Metrohom, USA). Concentrations of the partial pressure of carbon dioxide (pCO_2), the saturation states of calcite (Ωcalc.) and aragonite (Ωarag.) and the concentrations of carbonate (CO_3^{2-}) and bicarbonate (HCO_3^-) were subsequently calculated from the AT, pHT and temperature measurements (Supporting File Table S1) with constants from Mehrbach et al. (1973), as adjusted by Dickson and Millero (1987).

2.2. Sample processing, condition and meat index

After 38 days of treatment exposure, *T. militaris* were measured with Vernier callipers up to the nearest 0.01 mm and fresh weight (Fw) of the whole body including shell and calcareous operculum was measured using a top pan balance with precision of ± 0.01 g (Ohaus Navigator). Turban snails were frozen at −80 °C prior to processing for analysis. Three snails were randomly selected from each mesocosm, generating a total of nine for each treatment. After thawing, the shell of each snail was cracked using a bench vice to allow removal of the foot muscle and viscera. After de-shelling, the soft-body without operculum (Fwo) and foot muscle after removal of the gonad and digestive gland (GDg) were weighed. The weight of all the shell fragments was also measured (Sww) using an analytical balance with precision of ± 0.0001 g (Mettler Toledo, model ML 204/01). The condition index of the turban snails were calculated as Fwo/Sww as described in Vasconcelos et al. (2008) and the meat yields of the snails were calculated as (Fwo – GDg)/Fw) x 100 (Okumus and Stirling, 1998).

2.3. Proximate analysis

Proximate composition analysis of individual snails (n = 9 per treatment, 3 per tank) was carried out as described in Ab Lah et al. (2017a). Each sample of 1 g foot flesh (w/w) was dried in the oven at 60 °C for up to 48 h. For the ash content, the dry samples were then transferred to a muffle furnace (MTI Corporation, model KSL 1700X) at 550 °C for 4 h. Biuret Assay was used to estimate the total protein by

Table 1
Proximate composition of the foot tissue of *Turbo militaris* a) Percent of fresh weight (mean ± standard error from n = 9 snails from 3 replicate mesocosms per treatment); and b) Statistical results based on PERMANOVA analysis. Significant *P* value are in bold.

a) Treatments	Current pCO_2, 22 °C	Current pCO_2, 25 °C	Future pCO_2, 22 °C	Future pCO_2, 25 °C
Condition index	60.64 ± 6.65	60.93 ± 8.60	57.40 ± 8.34	59.87 ± 9.30
Meat Yield	22.85 ± 3.00	22.57 ± 4.75	21.41 ± 3.57	20.09 ± 3.68
Moisture	70.94 ± 2.34	71.76 ± 1.39	72.21 ± 2.65	71.45 ± 1.57
Ash	2.41 ± 0.48	2.73 ± 0.36	2.91 ± 0.91	2.66 ± 0.75
Protein	13.80 ± 4.33	15.01 ± 2.52	13.98 ± 2.61	15.92 ± 2.11
Lipid	5.80 ± 0.50	6.81 ± 0.94	6.47 ± 0.74	6.44 ± 1.17

b)	Temperature		pCO_2		Temperature x pCO_2		Mesocosm (Temperature x pCO_2)	
	Pseudo F	*P*-value	Pseudo F	P-value	Pseudo F	P-value	Pseudo F	*P*-value
Condition index	1.42	0.26	0.80	0.37	1.87	0.21	2.25	0.06
Meat yield	0.30	0.63	1.81	0.23	0.13	0.76	1.47	0.22
Moisture	< 0.01	1	0.47	0.55	0.96	0.38	1.41	0.23
Ash	0.01	0.95	0.43	0.63	0.56	0.56	3.12	**0.02**
Protein	1.51	0.27	0.07	0.83	0.09	0.80	2.82	**0.02**
Lipid	1.96	0.21	0.45	0.52	1.33	0.29	1.17	0.37

measuring the absorbance of NaOH digested protein samples at 550 nm on a spectrophotometer (Perkin Elmer, model Victor X4). Lipids were extracted from each sample according to the methods of Woodcock and Benkendorff (2008) based on the original method by Bligh and Dyer (1959) in chloroform:methanol (solvent ratio 1:2).

2.4. Analysis of fatty acid composition

Fatty acid methyl esters (FAMEs) of the lipid extracts were prepared as outlined in Ab Lah et al. (2017a) and Valles-Regino et al. (2015). The lipid extracts were briefly heated in 0.5 M solution of sodium hydroxide (NaOH) in methanol then methylated using 2 ml of 14% boron trifluoride (BF_3) in methanol followed by hexane extraction. The upper hexane layer was transferred to a separate auto-sampler vial for Gas Chromatography (GC) injection on an Agilent 6890 N with flame ionization detector. FAMEs were identified by comparison to reference standard FAMEs test mix (Sigma) with supplementary analysis as required for identification of unmatched peaks using GCMS analysis (Agilent 6890) coupled to an Agilent 5973 mass selective detector and comparison to the mass spectral library (WILEY 275 and NIST98).

2.5. Analysis of essential elements

For elemental analysis, foot tissue sub-samples (1 g) were sent to the Environmental Analysis Laboratory (EAL), Southern Cross University (National Association of Testing Authorities, Australia (NATA); Accreditation Number 14960). Samples of fresh weighed turban snail foot tissues were digested in a mixture of Nitric acid (HNO_3, 25%) and Hydrochloric Acid (HCl, 75%) (1:3, v/v) subjected to hot-block (Hot-Block; Environmental Express, South Carolina, U.S.A) acid digestion procedure (APHA, 2012). All elemental concentrations were determined on a wet weight basis and analysed on an inductively-coupled plasma (ICP) spectrometer (NexION 300 D series) with ESI SC-FAST Auto Sampler (Perkin Elmer, Waltham, Massachusetts, U.S.A.).

2.6. Statistical analysis

The data are expressed as means ± standard error. For statistical analysis, the data were tested using nonparametric permutational analyses in PRIMER v. 6 + PERMANOVA add-on (PRIMERe Pty. Ltd.). Three factor PERMANOVAs were run with main effects being temperature and pCO_2, with replicate mesocosms (random) nested within temperature and pCO_2. Analyses were undertaken on Euclidean distance matrices using 9999 permutations on the full model. Univariate PERMANOVAs were used to test the condition index, the meat yield, each of the proximate component (protein, ash, moisture and total lipid), the percent of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), Omega–3 (n–3) and Omega–6 (n–6) fatty acids. Additionally, univariate analyses PERMANOVAs were also performed on the n-3/n-6 ratio, as well as the three most dominant PUFAs arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosapentaenoic (DPA), as well as the total dimethyl acetal aldehyde (DMA) composition. Multivariate PERMANOVA was used to investigate the overall composition of fatty acids. Three separate multivariate PERMANOVAs were also used to assess the minerals (macro elements, micro elements and toxic elements) given they were found in different orders of magnitude within in the tissue. For the mineral compositions, the data were normalised to the same scale prior to generating the Euclidean distance similarity matrix. Principle component ordination (PCO) was undertaken to visually display the differences between treatments in fatty acids and mineral elements, with vector overlay using Pearson rank correlation. Additional univariate PERMANOVAs were used to test for differences in the quantity of calcium, zinc and iron in the foot tissue. In all analyses, statistical significance was set at $\alpha = 0.05$.

3. Results

The condition index was similar in *T. militaris* under all treatment conditions (ranging from 57% to 61% flesh, relative to shell weight), with no significant differences due to ocean acidification, warming or their interaction between these two factors (Table 1). The meat yield ranged from 20% to 23% of the total flesh weight (Table 1a). The average percent meat yield was 2.7% higher in current conditions compared those subject to ocean acidification (Table 1a). However, there were no significant effects of pCO_2 or temperature on meat yield and no significant interaction between these (Table 1b).

Overall, moisture content of the foot tissue ranged from 71% to 73%, whilst ash varied from 2.41% to 2.91% (Table 1a). Protein varied between 13.8% and 15.9% of wet weight basis, whereas lipid ranged from 5.80 to 6.81% (Table 1a). None of these proximate components varied significantly among treatments (Table 1b), although there were significant random tank effects for protein and ash.

The fatty acid composition of the lipid extracts from the foot tissue of *T. militaris* are presented in Table 2. The SFAs in *T. militaris* under current and future ocean conditions were dominated by palmitic acid (C16), followed by lignoceric acid (C24) and stearic acid (C18) (Table 2). Amongst the MUFAs, oleic acid (C18:1(n–9)) had the highest concentration (Table 2a). Docosapentaenoic acid (DPA) (C22:5(n–3)), AA (C20:5(n–6)), docosadienoic acid (C22:2(n–6)) and EPA (20:5(n–3))

Table 2
Fatty acid composition of *Turbo militaris* a) mean percent of total fatty acids ± standard deviation from n = 9 snails from 3 replicate mesocosm per treatment; and b) Statistical results for the univariate and multivariate PERMANOVAs with two factors, temperature and pCO_2 induced acidification, on the fatty acid composition.* Significant *P* values are in bold.

a) Fatty acid	Trivial name	Temperature (22 °C)		Temperature (25 °C)	
		Current pCO_2	Future pCO_2	Current pCO_2	Future pCO_2
C14:0	Myristic	1.07 ± 0.04	1.02 ± 0.05	0.81 ± 0.03	0.83 ± 0.03
C15:0	Pentadecanoic	1.42 ± 0.03	1.46 ± 0.06	1.47 ± 0.02	1.43 ± 0.06
C16:0	Palmitic	19.61 ± 0.29	20.34 ± 0.54	19.55 ± 0.25	20.09 ± 0.26
C17:0	Margaric	2.69 ± 0.06	2.78 ± 0.11	3.56 ± 0.35	2.94 ± 0.16
C18:0	Stearic	5.02 ± 0.09	4.84 ± 0.14	5.22 ± 0.14	5.13 ± 0.11
C24.0	Lignoceric	6.64 ± 0.25	6.98 ± 0.25	7.41 ± 0.35	7.60 ± 0.29
Total SFA		36.45 ± 0.43	37.45 ± 0.60	38.01 ± 0.40	38.03 ± 0.43
C16:1	Palmitoleic	0.75 ± 0.05	0.87 ± 0.06	0.71 ± 0.04	0.89 ± 0.04
C18:1(n-9)	Oleic	7.10 ± 0.14	7.21 ± 0.22	6.63 ± 0.12	7.16 ± 0.19
C20:1(n-9)	Eicosenoic	2.30 ± 0.08	2.15 ± 0.07	2.48 ± 0.12	2.37 ± 0.11
C22:1(n-9)	Erucic	0.18 ± 0.03	0.13 ± 0.02	0.09 ± 0.01	0.13 ± 0.03
Total MUFA		10.33 ± 0.14	10.36 ± 0.24	9.91 ± 0.17	10.54 ± 0.17
C18:2(n–6)	Linoleic (LA)	2.37 ± 0.06	2.59 ± 0.12	2.42 ± 0.09	2.39 ± 0.18
C18:3(n–3)	a–Linoleic (ALA)	1.53 ± 0.07	1.55 ± 0.10	1.38 ± 0.04	1.31 ± 0.20
C20:3(n–3)	Eicosatrienoic (ETA)	0.17 ± 0.03	0.14 ± 0.02	0.12 ± 0.02	0.15 ± 0.02
C20:4(n–6)	Arachidonic (AA)	12.80 ± 0.29	13.46 ± 0.20	13.66 ± 0.24	13.94 ± 0.23
C20:5(n–3)	Eicosapentaenoic (EPA)	3.24 ± 0.14	2.87 ± 0.25	2.25 ± 0.15	2.01 ± 0.11
C22:6(n–3)	Docosahexaenoic (DHA)	0.45 ± 0.04	0.46 ± 0.05	0.36 ± 0.04	0.34 ± 0.06
C22:5(n–3)	Docosapentaenoic (DPA)	11.19 ± 0.30	10.41 ± 0.26	10.04 ± 0.20	9.70 ± 0.25
C20:2	Eicosadienoic	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.02	0.07 ± 0.00
C22:2	Docosadienoic	6.24 ± 0.31	6.40 ± 0.34	6.71 ± 0.14	6.98 ± 0.24
N–3		16.59 ± 0.37	15.43 ± 0.43	14.12 ± 0.39	13.52 ± 0.39
N–6		15.17 ± 0.32	16.05 ± 0.18	16.03 ± 0.23	16.34 ± 0.16
N–3:n–6		1.10 ± 0.04	0.96 ± 0.03	0.88 ± 0.03	0.83 ± 0.03
Total PUFA		38.05 ± 0.30	37.95 ± 0.46	37.01 ± 0.27	36.90 ± 0.35
Others:					
2–octylcyclo–propanedecanoic		0.39 ± 0.07	0.28 ± 0.05	0.33 ± 0.08	0.28 ± 0.05
Dimethyl acetal aldehydes:					
Hexadecane–1–al		0.27 ± 0.04	0.27 ± 0.36	0.22 ± 0.03	0.32 ± 0.02
Heptadecan–1–al		1.66 ± 0.09	1.48 ± 0.07	1.66 ± 0.09	1.52 ± 0.05
Octadecan–1–al		12.36 ± 0.25	11.85 ± 0.43	12.36 ± 0.25	12.05 ± 0.34
Nonadecan–1–al		0.10 ± 0.02	0.09 ± 0.01	0.10 ± 0.02	0.08 ± 0.02
Total DMA		14.39 ± 0.28	14.03 ± 0.32	14.28 ± 0.35	13.97 ± 0.38

b)	Temperature		pCO_2		Temperature x pCO_2		Mesocosm (temperature x pCO_2)	
	Pseudo F	*P* value	Pseudo F	*P* value	Pseudo F	*P* value	Pseudo F	*P* value
SFA	5.95	**0.02**	0.79	0.38	0.66	0.44	1.83	0.12
MUFA	0.20	0.66	2.06	0.19	1.72	0.22	1.29	0.30
PUFA	6.1	**0.004**	0.005	0.94	0.03	0.89	1.94	0.09
N–3	26.92	**0.0006**	4.05	0.08	0.48	0.50	1.22	0.33
N–6	10.02	**0.02**	5.72	**0.04**	0.42	0.52	1.66	0.16
N–3/n–6 ratio	28.05	**0.0007**	6.72	**0.03**	0.90	0.36	1.15	0.37
C20:5(n–3) (EPA)	27.35	**0.003**	1.32	0.27	0.005	0.94	1.23	0.32
C22:5(n–3) (DPA)	13.34	**0.0007**	2.89	0.13	0.30	0.63	1.34	0.27
C20:4(n–6) (AA)	3.68	0.09	1.28	0.28	0.03	0.86	4.37	**0.003**
Total DMAs	0.012	0.97	0.65	0.44	0.34	0.58	3.26	**0.01**
Overall fatty acid composition	4.05	**0.001**	1.21	0.28	0.41	0.89	1.76	**0.01**

were identified as the primary PUFAs (Table 2a). The n–3 fatty acids consist of α–linolenic acid, eicosatrienoic acid, EPA, DPA and docosahexaenoic acid (DHA) and n–6 fatty acids: linoleic acid, AA, eicosadienoic acid and docosadienoic acid (Table 2a). A cyclopropane-containing fatty acid (2–octylcyclo–propanedecanoic) was also found in the foot tissue of *T. militaris*. A series of DMAs were identified in the lipid composition, including hexadecane–1–al, heptadecan–1–al, octadecan–1–al and nonadecan–1–al derivatives (Table 2a).

The fatty acid composition in all treatments was dominated by SFA and PUFAs, but contained a relatively small proportion of MUFAs (Fig. 1a). There were no significant differences across all ocean treatment conditions in the percentage of monounsaturated fatty acids (MUFA) (Table 2b). PUFAs significantly decreased under ocean warming (Fig. 1a), but were not affected by acidification treatments (Table 2b), whilst exposure to higher temperature resulted in a significant increase in the proportions of SFA (Fig. 1a, Table 2b). The proportion of n–6 PUFAs was significantly reduced under both elevated temperature and pCO_2 treatments (Table 2b, Fig. 1b and c), whereas only temperature affected the percentages of n–3 (Fig. 1b). A greater decrease in n–6 fatty acids resulted in significant differences in the ratio of the n–3/n–6 PUFAs (Table 2b, Fig. 1c). Further univariate analysis found that EPA and DPA were significantly reduced by ocean warming, but not pCO_2 induced acidification (Table 2b). At elevated temperatures, EPA and DPA were significantly decreased (Fig. 1b), in contrast to AA, which showed a non-significant positive trend (Fig. 1b) and significant variation between mesocosms (Table 2b). There were also differences in the overall fatty acid composition in the foot tissue of *T. militaris* exposed to future ocean conditions for 38 days (Table 2b). Multivariate PERMANOVA demonstrated that despite significant mesocosm effects, ocean warming, but not acidification had a significant effect on the overall fatty acid composition, with no interaction between temperature and acidification (Table 2b). In the PCO plot

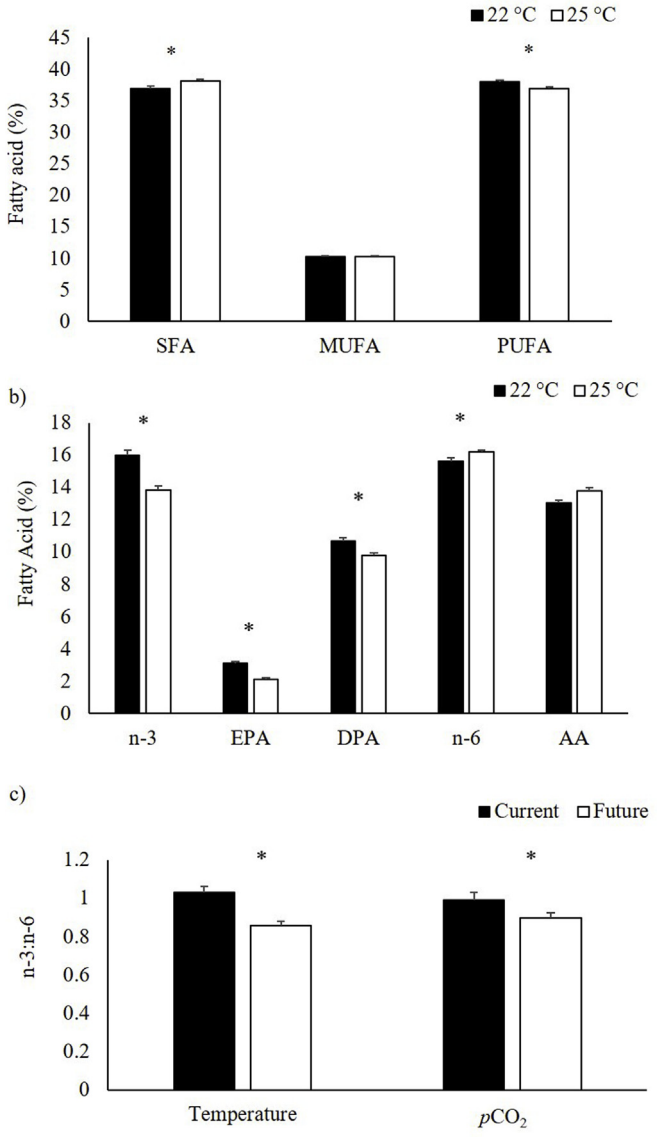

Fig. 1. The fatty acid profile of *Turbo militaris* foot tissue showing significant treatment effects after exposure to current and future ocean climate conditions for 38 days. Proportion of: a) saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids; and b) composition of n–3 and n–6 PUFAs, including eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and arachidonic acid (AA), after exposure to different temperatures; and c) the ratio of n-3 to n-6 PUFAs after exposure to current (22 °C) and future (25 °C) temperatures or current (~393 ppm) and future (~830 ppm) pCO_2-induced acidification. * Significant difference between treatments, with separate univariate PERMANOVAs performed for each class of fatty acid ($P < 0.05$). Each bar represented the mean ± SE (n = 18 snails from 6 replicate mesocosms per temperature or CO_2 treatment).

(Fig. 2), the significant effect of temperature on fatty acid composition is apparent, and characterized by higher EPA, DPA and myristic acids (Fig. 2). Vector overlay using Pearson rank correlation suggests that the snails held at 25 °C show a tendency towards higher AA and lignoceric acids (Fig. 2).

Dimethyl acetal aldehydes (DMAs) in foot tissue of *T. militaris* were only detected at low levels within all treatments (less than 2%), except for octadecan–1–al which comprises more than 11% of the total lipid composition (Table 2a). Univariate analysis revealed no significant difference in the percentage of DMAs after the turban snails were exposed to the four different ocean conditions (Table 2b).

Among the macroelements, the most abundant mineral in *T. militaris* foot tissue was sulphur (S), followed by sodium (Na), potassium (K) and phosphorous (P). Macro-elemental composition was not influenced by ocean warming or pCO_2 induced acidification (Table 3b). Iron (Fe), followed by zinc (Zn) and copper (Cu) were the dominant microelements detected in *T. militaris* foot tissue (Table 3a). For the overall composition of macro, micro and trace elements, PERMANOVA analysis found no significant temperature or pCO_2 effects (Table 3b), but there were some random differences in microelements between mesocosms (Supplementary File, Fig. S1). In relation to the individual microelements, Fe and Zn appear to be lower in current ocean temperature and pCO_2 conditions (Table 3a). Univariate analysis confirmed that zinc (Fig. 3a), but not iron (Fig. 3b), was significantly higher by 2 mg/kg at elevated temperature (25 °C) relative to 22 °C (Table 3). Within temperature treatments, calcium showed a trend towards lower quantities in the flesh under future pCO_2 conditions (Fig. 3c), but this was not significant. However, there was significant variation in calcium (Ca) between mesocosms within treatment groups (Table 3b).

4. Discussion

The results presented here reveal the potential impacts of ocean warming and acidification on the nutritional quality and trace element composition of the edible turban snail *T. militaris*. This study demonstrates that predicted future ocean temperatures may significantly affect the fatty acid composition of *T. militaris*. In particular, warmer water causes an overall reduction in essential PUFAs, including the omega–3 PUFAs EPA and DPA. Under the elevated temperature and pCO_2, there was a decrease in the ratio of n–3/n–6 resulting mainly from the reduction of EPA, DPA and increase of AA. Condition index, meat yield, proximate composition (ash, moisture, lipid and protein) and mineral elements were not negatively affected by future ocean conditions.

Elevated temperatures are known to increase the metabolic rate (Brockington and Clarke, 2001; Ganser et al., 2015; Li et al., 2007), and impact biochemical processes in marine invertebrates, which is expected to increase the metabolic 'cost of living' for organisms outside their thermal optima (Somero, 2002). However, we found no significant effect on the condition index, meat yield or proximate components of *T. militaris* after exposure to warmer waters for 38 days. Even though proteins play a vital role in the stress response (e.g. Sanders, 1988; Somero, 2002) and can potentially be catabolised from endogenous sources to supply essential amino acids and energy in molluscs (Hochachka, 1983; Barber and Blake, 1885; Kreeger et al., 1995), we found no significant effects of elevated temperature or pCO_2 induced acidification on the protein content of the flesh of *T. militaris*. This outcome is in contrast to recent findings for the predatory gastropod *D. orbita,* which showed large reductions in the flesh protein content (Tate et al., 2017). However, in a previous study on the clam *R. philippinarum* significantly higher protein levels were found at the extreme temperature of 34 °C compared to 22 °C (Anacleto et al., 2014). In another study on the ornamental red cherry shrimp, *Neocaridina heteropoda heteropoda*, no difference in protein content was found after exposure to three different ocean temperature levels over 90 days (Tropea et al., 2015). This suggests the effects of elevated temperature on the protein content of marine invertebrates may be species specific and could depend on a number of other factors, such as temperature tolerance, metabolic rates and nutritional status. In our recent studies, the preferred temperature of *T. militaris* was found to be 22–24 °C, with a critical thermal maxima closer to 30 °C (Ab Lah et al., 2017b). Therefore, *T. militaris* may experience minimal thermal stress at the future predicted elevated water temperatures of 25 °C. Alternatively, higher metabolic rates may have resulted in higher feeding rates at the elevated temperature, thus not requiring mobilisation of stored protein. Increased rates of photosynthesis under elevated temperature and pCO_2 could be beneficial to the growth rates of macroalgae (Connell and Russell, 2010; Hepburn et al., 2011), thus providing sufficient nutrition for grazers under the

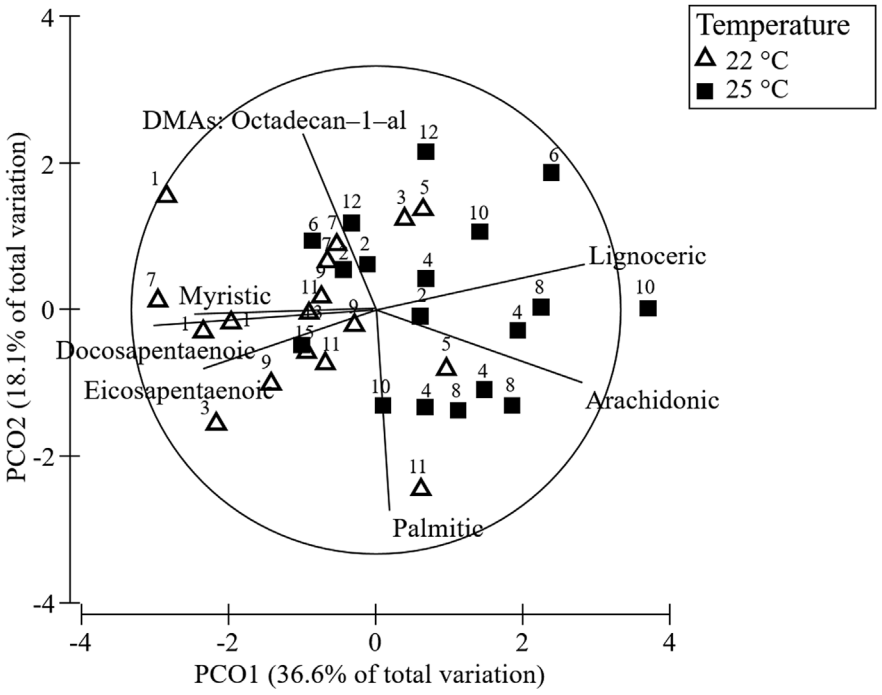

Fig. 2. Principle component ordination (PCO) of the fatty acid profiles from the foot tissue of *Turbo militaris* after exposure to current and future elevated water temperature (with mesocosm numbers as labels) based on a Euclidian distance similarity matrix of the percent composition data with vector overlay from Pearsons correlation > 0.7.

Table 3
Elemental compositions in the foot tissue of *Turbo militaris* after exposure to 38 days of different ocean condition treatments: a) Mean ± standard deviation of each element as g/kg fresh weight (FW) (n = 9 snails from 3 replicate mesocosms per treatment); and b) Summary of the statistical results for the multivariate and univariate analyses with three factor, temperature and pCO_2 (fixed) and replicate mesocoms (random) on the composition of microelements, macroelements, toxic elements (multivariate) and iron, zinc as well as calcium (univariate). Significant *P* values in bold.

a) Elements	Temperature (22 °C)		Temperature (25 °C)	
	Current pCO_2	Future pCO_2	Current pCO_2	Future pCO_2
Macroelements (g/kg FW)				
Na	4.34 ± 0.37	4.58 ± 0.23	4.12 ± 0.19	4.20 ± 0.26
K	2.24 ± 0.11	2.30 ± 0.07	2.22 ± 0.09	2.30 ± 0.11
Ca	0.46 ± 0.09	0.39 ± 0.02	0.51 ± 0.08	0.40 ± 0.06
Mg	0.79 ± 0.04	0.83 ± 0.02	0.77 ± 0.02	0.75 ± 0.03
P	1.12 ± 0.04	1.15 ± 0.03	1.12 ± 0.03	1.13 ± 0.05
S	8.32 ± 0.28	8.60 ± 0.22	8.44 ± 0.22	8.47 ± 0.38
Microelements (mg/kg FW)				
Fe	37.31 ± 4.15	41.63 ± 4.77	47.42 ± 9.17	46.08 ± 7.74
Zn	12.65 ± 0.41	13.92 ± 0.29	15.24 ± 0.90	15.34 ± 0.73
Cu	3.01 ± 0.25	3.00 ± 0.29	3.47 ± 0.29	3.01 ± 0.39
Co	0.09 ± 0.02	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.01
Se	0.16 ± 0.02	0.18 ± 0.01	0.18 ± 0.01	0.16 ± 0.01
Toxic elements (mg/kg FW)				
Mn	0.41 ± 0.03	0.47 ± 0.03	0.41 ± 0.04	0.41 ± 0.04
Al	1.60 ± 0.31	3.15 ± 1.43	1.60 ± 0.22	2.05 ± 0.47
As	10.75 ± 1.22	12.92 ± 1.51	11.92 ± 0.93	10.96 ± 0.99
Cd	0.06 ± 0.01	0.06 ± 0.004	0.06 ± 0.01	0.06 ± 0.01
Cr	0.47 ± 0.04	0.54 ± 0.06	0.46 ± 0.03	0.49 ± 0.06
Ni	0.09 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.25 ± 0.13
Pb	0.32 ± 0.03	0.40 ± 0.03	0.43 ± 0.13	0.28 ± 0.03
Ag	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.002	0.01 ± 0.002
Hg	0.01 ± 0.001	0.01 ± 0.002	0.01 ± 0.002	0.01 ± 0.001

b)	Temperature		pCO_2		Temperature x pCO_2		Mesocosm (temperature x pCO_2)	
	Pseudo F	*P* value	Pseudo F	*P* value	Pseudo F	*P* value	Pseudo F	*P* value
Macroelements	1.00	0.39	0.30	0.92	0.38	0.85	1.35	0.18
Microelements	1.62	0.06	0.81	0.70	0.88	0.61	2.31	**0.001**
Toxic elements	0.69	0.78	0.81	0.64	0.75	0.74	0.89	0.71
Iron	1.06	0.34	0.0001	0.99	0.25	0.60	2.13	0.06
Zinc	19.71	**0.002**	2.70	0.15	1.48	0.26	0.43	0.90
Calcium	0.94	0.50	0.31	0.95	0.75	0.64	3.80	**0.005**

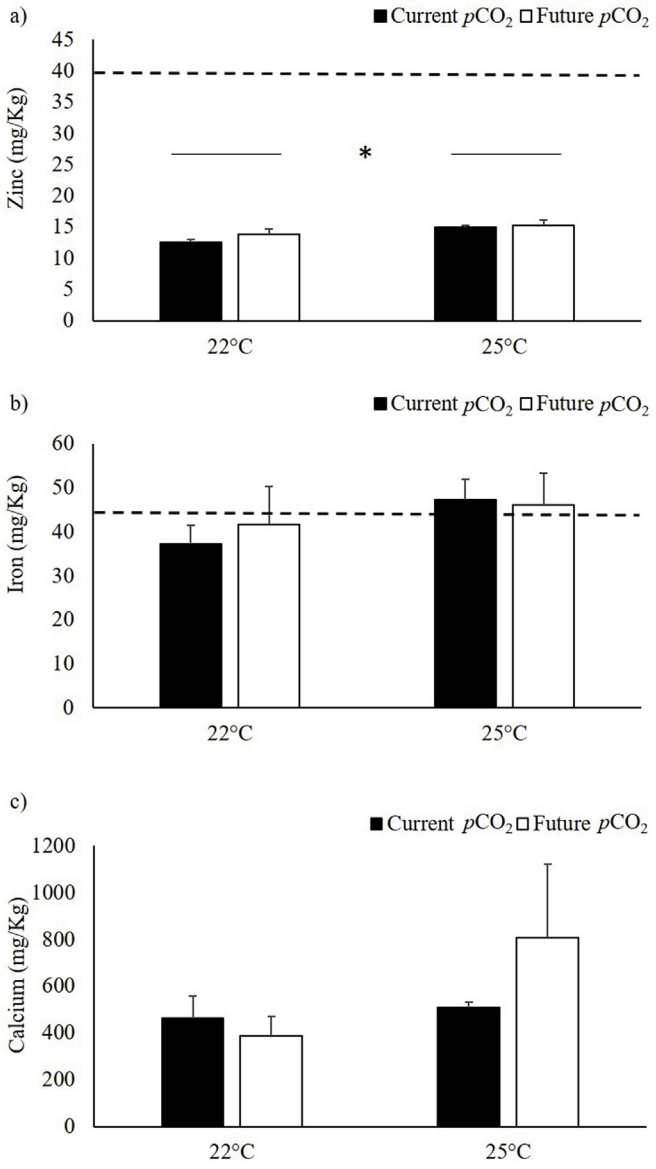

Fig. 3. The effects of ocean climate change stressors on minerals in the foot tissue of *Turbo militaris*. The quantity of: a) zinc, b) iron and c) calcium (wet weight, mg/kg), after 38 days exposure to temperature and pCO_2 treatments with the line showing the acceptable level for human intake based on mg/day. Each bar represents the mean ± SE (n = 9 snails from 3 replicate mesocosms per treatment).

ocean conditions trialled in this study. The turban snails were allowed to graze *ad libitum* on algae growing naturally in the mesocosms during our experiment. However, protein and ash were found to vary between mesocosms within treatments, which may reflect random variation in the specific algal composition naturally settling in the mesocosms. Nevertheless, results from a previous study on diatoms and copeopods have demonstrated that negative impacts of ocean acidification on the macromolecules of primary producers can become magnified in primary consumers, with strong implications for ocean food webs (Rossoll et al., 2012). Manipulative studies that simultaneously examine nutritional changes in macroalgae and their grazers under future conditions would be worthwhile.

After proteins, lipids are the next most dominant organic component of the Turbinidae flesh (Ab Lah et al., 2017a). Lipids are structural components of all cell membranes and also provide important sources of stored energy for growth and reproduction (Parrish, 2013; Valles-Regino et al., 2015). We found no significant effects of elevated temperature or pCO_2 induced acidification on the total lipids. Again, this is in contrast to previous findings on the predatory gastropod *D. orbita,* in which there was a significant reduction (almost 50%) in total lipids in the foot tissue after exposure to similar regimes of elevated water temperatures (Valles-Regino et al., 2015). This suggests that the herbivorous turbans may be more resistant to changes in lipid storage than the carnivorous *D. orbita*. However, lower lipid content was also found under higher temperature conditions for the grazing sea urchin *Haliotis tuberculata* (Hernandez et al., 2013). Su et al. (2006) have suggested that abalone may have reduced ability to convert non-lipid into lipid at higher temperatures, but this needs to be confirmed in manipulative experiments with controlled diets. Overall, our results on the proximate composition of *T. militaris* imply that future oceanic conditions may not be detrimental to the amount of protein or lipid per tonne harvested from herbivorous gastropods, providing the availability and nutritional quality of their macroalgal diet is not negatively impacted.

Despite no significant changes in the total lipids, we found significant differences in fatty acid composition of the foot tissue of *T. militaris*, which has implications for seafood quality. Temperature, but not ocean acidification strongly affected the lipid composition, with an increase in the percentage of SFA and a corresponding decrease in PUFA. These results are consistent with the previous study on *D. orbita*, which also found the percentage of PUFAs was reduced at elevated temperatures, irrespective of pCO_2 conditions (Valles-Regino et al., 2015). Similar reductions in PUFAs were observed at higher temperatures in the mangrove oyster *Crassostrea rhizophorae* (Martino and Cruz, 2004) and clams *R. decussatus* and *R. philippinarum* (Anacleto et al., 2014). An increase in SFA content after exposure to elevated water temperature was also found for the Jade Tiger hybrid abalone from Australia (Mateos et al., 2010). As the FAMEs process hydrolyses and esterifies fatty acids from all stored and structural glycerides and phospholipids, the observed changes in the relative proportions of SFA and PUFA imply compensatory changes in fluidity of the lipid bilayer of cell membranes in response to temperature, a process known as homeoviscous adaptation (HVA) (Dooremalen et al., 2011). With decreasing temperature, the level of PUFAs increase in order to keep the membrane fluidity constant, whereas, the increase of SFAs under higher temperatures will avoid membrane “hyperfluidization” if the temperature exceeds the optimal thermal tolerance (Anacleto et al., 2014; Dooremalen et al., 2011). More specifically Hall et al. (2002) showed a strong relationship between cell membrane fluidity and EPA composition in the gill of *P. magellanicus*. Thus the reduced amount of EPA in *T. militaris* tissue under elevated temperature is consistent with previous studies showing high EPA levels in the bivalves *Placopecten magellanicus* (Hall et al., 2002) and *Crassostrea virginica* (Pernet et al., 2007) acclimatised to lower temperature, supporting the hypothesis that temperature induced restructuring of PUFA composition occurs as a consequence of adaptation of the membrane fluidity balance in marine molluscs.

Temperature not only affected the PUFAs, but also the relative proportions of long chain n–3 and n–6 fatty acids in the turban snails. The n–3 and n–6 PUFAs are known for their beneficial effects on human health since they are important for reducing cholesterol levels and coronary heart disease, as well as helping to prevent arteriosclerosis and inflammation (Mahaffey, 2004; Mahaffey et al., 2008; Simopoulos, 2002). Valles-Regino et al. (2015) found that ocean warming, but not acidification, affected the composition of n–3 and n–6 PUFAs in *D. orbita*, whereas in this study both ocean warming and acidification had independent significant effects on the relative proportion of n–6 PUFAs in *T. militaris*, while n–3 was only affected by elevated temperature. The ratio of n–3/n–6 fatty acids was reduced from 1.1 under current conditions to less than 1 after exposure to the future ocean treatments for 38 days. The ratio of n–3/n–6 fatty acids is a good indicator for measuring the nutritional benefits of fatty foods and the Department of Health (UK) guidelines recommend a ratio between 1 and 4 (Milinsk

et al., 2003). This study demonstrates that future-ocean warming and acidification can alter the ratio of n–3/n–6 fatty acid below the optimum value, primarily resulting from a deficiency in healthful n–3 fatty acid such as EPA, DHA and DPA.

Molluscs are also a good source of minerals and have been considered as suitable bio-indicators for trace element availability in the surrounding environment (Cravo and Bebianno, 2005; Huang et al., 2008). Ocean warming and acidification may influence trace element availability and uptake, as both these stressors can affect the feeding rates of organisms (Baines et al., 2005; Lopez et al., 2010; Nesto et al., 2007; Sokolova et al., 2012). Shell dissolution under acidified conditions could also mobilize elements from the shell matrix, which in turn could influence the balance of other inorganic ions in the organism tissues (Gutowska et al., 2010; Melzner et al., 2009; Pörtner et al., 2004). Despite the well-known effects of ocean acidification on calcium carbonate in the shells of molluscs (Parker et al., 2013), we found no significant differences in Ca in the foot tissue of *T. militaris* after exposure elevated pCO_2 or temperature. However, Zn significantly increased in the flesh of *T. militaris* exposed to elevated ocean temperatures and similar trends were found for Fe. Iron and Zn are necessary for human health as these micronutrients are essential for human growth, development, and maintenance of the immune system (Walker et al., 2005). But an excessive intake of Fe and Zn in the human diet is potentially harmful to human health (NHMRC, 2015). The turban snails held under future conditions contain up to 15 mg/kg of Zn in their tissue, which does not exceed the recommended upper intake for an adults (> 18 years old) of 40 mg/day (NHMRC, 2006), based on a typical serving of ~0.3 kg (=3 snails). Under future ocean temperature the average of iron composition in the foot tissue of *T. militaris* could exceed the tolerable upper intake for human consumption (45 mg/day), but only if a very large serving was consumed (NHMRC, 2006). Nevertheless, the effects of ocean warming and acidification on the availability of metal ions in seafood should be considered, due to the potential bioaccumulation of metals through trophic system interactions, which could pose serious risk to humans.

5. Conclusion

Overall, this study found that *T. militaris* appears to be relatively resilient to elevated temperature and pCO_2 conditions predicted from future ocean climate change models, with no significant changes to the condition index, meat yield, overall proximate composition or trace element composition. However, changes in the fatty acid composition were detected that are consistent with physiological adaptation to elevated temperatures. The increases in SFA and decreases in n-3 PUFAs in snails held under elevated temperature compared to snails held under current ocean temperature conditions could have negative consequences for seafood quality and the associated benefits to human health. Zn concentrations significantly increased with ocean warming, but remained within safe levels for human consumption. The other trace elements remain similar between all treatments regardless of temperature or acidification levels. Given the potential changes to seafood nutritional quality from ocean warming and acidification, more work is needed to assess the impacts of ocean climate change on commercially-harvested marine species and the potential for trophic cascade.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.marenvres.2018.08.009.

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